

Serial No. 09/462,740



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Hiroshi MURAKAMI et al.
Serial Number : 09/462,740
Filed : April 5, 2000
For : Transgenic mammals
Art Unit : 1632
Examiner : Peter Paras, Jr.

DECLARATION

SIR:

I, Tamotsu SHIGEHISA, a citizen of Japan and residing at 10-5 Kasuga 4-chome, Tsukuba-shi, Ibaraki 305-0821, Japan, say and declare as follows:

1. I have received a Ph.D. from the College of Agriculture, Osaka Prefecture University, Japan, in 1987.
2. I have been working at the Research and Development Center (RDC), Nippon Meat Packers, Inc. (NMP) since 1971. I have been working in Animal Engineering Group of RDC, NMP since 1996. Presently, I am the president of a subsidiary company of NMP, the Animal Engineering Research Institute, Inc. specializing in development of transgenic pigs.
3. I am a member of Japan Society for Bioscience, Biotechnology and Agrochemistry, and the Japanese Society of Veterinary Science, and a board member of the Japanese Association for Animal Cell Technology.
4. I am a co-author of the references of the attached list.
5. I am one of the inventors of U.S. Serial Number 09/462,740 and familiar with the subject matter thereof.
6. I have read and understood the Office Action in connection with the above-identified Application. The Examiner asserts that the specification has not provided any guidance, teaching, or working examples relating to non-human

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mammals in xenotransplantation.

Hereinafter I will show the following: results of two series of transplantation experiments demonstrating that transgenic pigs generated according to the Application prevented hyperacute rejection (HAR) in pig-to-monkey xenotransplantation models and my comments.

(1) Dr. N. Fukushima, Assistant Professor, the First Surgery, Graduate School of Medicine, Osaka University, Japan, carried out one series of cardiac xenotransplantation experiments.

The hearts of nonimmunosuppressed rhesus monkeys were excised and replaced with those of either normal (n=2) or transgenic (n=3) pigs of this Application (i.e., orthotopic cardiac transplantation). The normal pig hearts were rejected in 77 min, whereas the transgenic pig hearts in 275 min. Such a significantly different survival period ($P<0.05$) demonstrated that the transgenic-pig hearts of the Application alleviated HAR.

It is generally known among skilled persons in the art that orthotopic transplantation results in shorter survival period than does heterotopic transplantation; the heterotopic transplantation is a method, wherein a recipient organ (monkey's heart) is kept intact and a donor organ (pig's heart) is transplanted commonly in the abdominal cavity of the recipient. The Artip's article cited by the Examiner relates to the heterotopic transplantation. It is a common sense in the art that the titers of natural antibodies resulting in HAR differ among individuals and species of the Old World monkeys and human beings, too.

Accordingly, simple comparison of the survival periods between the two studies by Fukushima and Artip is meaningless.

(2) Dr. T. Fujita, Assistant Professor, Plastic Surgery, Sapporo Medical University School of Medicine, Japan, carried out another series of skin transplantation experiments.

Pork skin grafts (0.02 inch in thickness) were harvested from the abdominal wall of a normal pig and the transgenic pig of this invention, and transplanted to the dorsal wall of a nonimmunosuppressed cynomolgus monkey. The skin grafts from the normal pig were rejected within two days, whereas those from the transgenic pig in not earlier than seven days after xenotransplantation. Histological examination demonstrated that the normal pig-skin grafts had typically been rejected by HAR, but that the transgenic pig-skin grafts had not been rejected by HAR but rejected like allograft (graft transplanted between the same species).

Accordingly, the transgenic pig skin of this invention prevented HAR.

These observations may relate to the specification of the Application (page 19, line 11-15): *Expression of hDAF was confirmed in the medium, small and capillary blood vessels of the transgenic pigs generated by introducing transgene comprising the pMCP promoter and hDAFcDNA. Besides, expression of hDAF was confirmed also in such organs as the peripheral nerves, skeletal muscle, and stratified squamous epithelia of the skin.*

7. It is well known that incorporation of transgene (DNA) to genome does not necessarily assure translation of protein or expression of function of said protein. The article by Kuipers et al. cited by the Examiner only shows existence of DNA in their transgenic pig genome and compares DNA levels between the pig and human control by slot-blot and FISH assays.

The specification of this Application, however, teaches the expression of hDAF protein (immunohistochemical assay) and the hDAF function (cell-lysis assay; i.e., prevention of complement activation). Moreover, levels of hDAF protein expressed at such organs as the heart and skin of this invention's transgenic pigs were quantitatively demonstrated to be comparable to those of man by ELISA (see Fig. 3 of *Molecular Reproduction and Development* 2002: 61; 302-311). Namely, the transgenic pigs of the Application highly express not only DNA but also functional protein of the hDAF in due organs.

Note:

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Literature

With a xenotransplantation combination between phylogenetically distant species (discordant combination like pig-to-primate), it is a common sense in the art that *natural antibodies of the recipient bind to endothelial cells lining the vessels of the donor organ; the complement cascade is triggered, and the endothelial cells are, as a result, "activated"* (page 450, column 2, paragraph 2, lines 2-5, *Immunology Today*, 1990:11; 450-456).

Accordingly, it is conceivable that description of functional protein of complement regulator (hDAF) expressed particularly at endothelial cells of transgenic-pig organs (i.e., the specification of this Application) makes remind persons in the art of the organs of this Application's transgenic pig as useful xenografts.

It is important to prevent complement activation at critical sites of to-be transplanted organs, particularly at the endothelial cells. The article by Artrip (page 176, column 1, paragraph 2) teaches the following: *"The resistance to hyperacute rejection was dependent on translocation of CRPs (complement restriction proteins) to endothelial cells and was short-lived following xenotransplantation.*

Current efforts use tissue-specific promoters such as von Willebrand factor or beta-actin for high level endothelial cell expression".

That is why the Application invented a novel transgene comprising the pMCP promoter and hDAFcDNA to express hDAF at the critical sites in the organ such as the endothelium. See the specification, page 5, line 2-4: *"The inventors succeeded in generating transgenic animals fulfilling the purposes with the promoter of the porcine complement inhibitor (pMCP) previously invented by the inventors.*

Conclusion

As shown above, the transgenic pigs generated according to the Application can overcome hyperacute rejection in pig-to-primate xenotransplantation.

8. I further declare:

that all statements made herein of my knowledge are true; and that all statements made on information and belief are believed to be true; and further

that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, of Title 18 of the United State Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated:

Apr. 11, 2003


Tamotsu SHIGEHISA

References

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5. H. Murakami, Y. Takahagi, M. Yoshimatsu, S. Miyagawa, T. Fujimura, K. Toyomura, T. Shigehisa, R. Shirakura and T. Kinoshita. Porcine MCP gene promoter directs high level expression of human DAF (CD55) in transgenic mice. *Immunobiol.* 1999/2000: 201; 583-597.
6. K. Toyomura, T. Fujimura, H. Murakami, T. Natsume, T. Shigehisa, N. Inoue, J. Takeda and T. Kinoshita. Molecular cloning of pig homologue of membrane cofactor protein (CD46). *Int. Immunol.* 1996: 9(6); 869-876.